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Structure–biocompatibility and transfection activity relationships of cationic polyaspartamides with (dialkylamino)alkyl and alkyl or hydroxyalkyl side groups

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ABSTRACT

A series of 14 cationic derivatives of poly(aspartic acid) *i.e.* cationic polyaspartamides with different (dialkylamino)alkyl and alkyl or hydroxyalkyl side groups was synthesized by nucleophilic addition on polysuccinimide. The resulting polyaspartamides have moderate amphiphilic properties. Relationships between the structure and ratio of side groups and *in vitro* properties of polyaspartamides, including their cytotoxic and membrane-damaging activity towards human cell lines, primary skin fibroblasts and erythrocytes, were established and discussed. Cationic polyaspartamides vary in their DNA-binding, condensing and nuclease-protecting characteristics depending on the concentration ratio of (dialkylamino)alkyl and alkyl or hydroxyalkyl side groups. Effective cell transfection was achieved upon polyaspartamide-mediated plasmid DNA delivery in serum-free medium in the presence of chloroquine. Effect of serum proteins adsorption onto polyaspartamide based polyplexes, and the role of concentration of polyplexes in culture medium in their colloidal stability and transfection process were demonstrated. Synthesized polyaspartamides are biocompatible and long-acting gene carriers, which are applied to cells after dilution and without washing, thus providing transfection level comparable to that of commercial transfection reagent.

1. Introduction

Synthetic polymers are an important tool in pharmaceutical engineering aimed at drug formulations with improved stability, availability and activity (Gaspar and Duncan, 2009). A range of different non-ionic and charged polymeric systems of synthetic or semisynthetic origin has been introduced to industrial and biomedical applications owing to their preferable physicochemical properties and compliance to reproducible production compared with natural polymers (Finch, 1983).

Polyacrylates are among the most pharmaceutically significant water soluble polymers synthesized by the radical polymerization

Abbreviations: α-MEM, minimum essential medium Eagle – alpha modification; B, *n*-butylamine; BSA, bovine serum albumin; CMC, critical micelle concentration; CPTA, 3-(carboxypropyl)trimethyl-ammonium chloride; DEAEMA, (diethylamino)ethyl methacrylate; DEE, 2-(diethylamino)ethylamine; DEP, 3-(diethylamino)-1-propylamine; DLS, dynamic light scattering; DMEM, Dulbecco's modified Eagle's medium; DME, 2-(dimethylamino)ethylamine; DMF, dimethylformamide; DMP, 3-(dimethylamino)-1-propylamine; DMSO, dimethyl sulfoxide; DNA, deoxyribonucleic acid; DNase I, deoxyribonuclease I; EDA, *N*-hydroxyethyl-ethylene diamine; EDTA, ethylenediaminetetraacetic acid; EGFP, enhanced green fluorescent protein; EtOAc, ethyl acetate; FBS, fetal bovine serum; FITC, fluorescein isothiocyanate; FTIR, fourier transform infrared spectroscopy; GPC, gel permeation chromatography; H, *n*-hexylamine; HD, hydrodynamic diameter; HEK-293, human embryonic kidney cell line; HLB, hydrophilic-lipophillic balance; HPLC, high performance liquid chromatography; HSF, human skin fibroblasts; HY, 6-amino-1-hexanole; HYD, hydrazine monohydrate; IC_{50}, half-maximal inhibitory concentrations; mRNA, messenger RNA; MTT, (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide); MW, average molecular weight; NMR, nuclear magnetic resonance; N/P ratio, molar ratio of amino groups in polycation to phosphate groups of pDNA; P, propylamine; P[Asp(DET)], poly(aspartic acid) grafted with diethylenetriamine; P[Glu(DET)], poly(glutamic acid) grafted with diethylenetriamine; PASP, poly(aspartic acid); PBLA, poly(β-benzyl-L-aspartate); PBS, phosphate buffered saline; PC-3, human adenocarcinoma cell line; PDI, polydispersity index; PEGMA, PEG-methacrylate; pDNA, plasmid deoxyribonucleic acid; PEG, polyethylene glycol; pEGFP-N2, plasmid vector encoding enhanced green fluorescent protein; PSI, polysuccinimide; siRNA, short interference ribonucleic acid; ζ, zeta potential.

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